The mediating role of brown fat and skeletal muscle measured by 18F-Fluorodeoxyglucose in the thermoregulatory system in young adults

Borja Martinez-Tellez[1,2†*], Mireia Adelantado-Renau[3†], Francisco M. Acosta[1], Guillermo Sanchez-Delgado[1], Antonio Martinez-Nicolas[4,5], Mariëtte R. Boon[2], Jose M. Llamas-Elvira[6,7], Vicente Martinez-Vizcaino[8,9], Jonatan R. Ruiz[1]

[1]PROFITH (PROmoting FITness and Health through Physical Activity) Research Group, Department of Physical Education and Sports, Faculty of Sport Sciences, University of Granada, Granada, Spain.

[2]Department of Medicine, division of Endocrinology, and Einthoven Laboratory for Experimental Vascular Medicine, Leiden University Medical Center, Leiden, the Netherlands.

[3]LIFE Research Group, University Jaume I, Castellon, Spain.

[4]Chronobiology Lab, Department of Physiology, College of Biology, University of Murcia, Mare Nostrum Campus. IUIE, IMIB-Arrixaca, Spain.

[5]Ciber Fragilidad y Envejecimiento Saludable (CIBERFES), Madrid, Spain.

[6]Servicio de Medicina Nuclear, Hospital Universitario Virgen de las Nieves, Granada, Spain.

[7]Biohealth Research Institute in Granada (ibs.GRANADA), Nuclear Medicine Department. Granada, Spain.

[8]Universidad de Castilla-La Mancha, Health and Social Research Center, Cuenca, Spain.

[9]Facultad de Ciencias de la Salud, Universidad Autónoma de Chile, Talca, Chile

*Corresponding author: borjammt@gmail.com

†These authors share first authorship

Address for correspondence: Borja Martinez-Tellez, MSc. Department of Physical Education and Sports, Faculty of Sport Sciences. University of Granada, Granada, Spain. Ctra de Alfacar s/n C.P. 18071. E-mail: borjammt@gmail.com; tel.: 0034 958242754; Fax: 0034 958244369

Acknowledgements

The authors would like to thank all the participants who took part in this investigation. This study is part of a Ph.D. Thesis conducted in the Biomedicine Doctoral Studies of the University of Granada, Spain. We are grateful to Alberto Quesada-Aranda for helping with the development of the Temperatus software (Free trial in http://profth.ugr.es/temperatus). We are grateful to Ms. Carmen Sainz-Quinn for assistance with the English language. This study was supported by the Spanish Ministry of Economy and Competitiveness, Fondo de Investigación Sanitaria del Instituto de Salud Carlos III (PI13/01393) and Retos de la Sociedad (DEP2016-79512-R), Fondos Estructurales de la Unión Europea (FEDER), by the Spanish Ministry of Education (FPU 13/04365), by the Fundación Iberoamericana de Nutrición (FINUT), by the Redes temáticas de investigación cooperativa RETIC (Red SAMID RD16/0022), by AstraZeneca HealthCare Foundation and by the University of Granada, Plan Propio de Investigación 2016, Excellence actions: Units of Excellence; Scientific Unit of Excellence on Exercise and Health (UCEES). M.A.R is supported by a Predoctoral Research Grant from UJI (PREDOC/2015/13). A.M.N was supported by the Ministry of Economy and Competitiveness, the Instituto de Salud Carlos III through CIBERFES (CB16/10/00239) and grant 19899/GERM/15 (co-financed by FEDER).

Declaration of competing financial interests

The authors declare they have no actual or potential competing financial interests.

ABSTRACT

BACKGROUND: Upon a cold exposure, brown adipose tissue (BAT) and skeletal muscles are activated as part of the thermoregulatory system, although the exact contribution of these tissues remains unknown. The personal level of environmental (Personal-ET) and wrist temperatures (WT) are measures of ambient and body temperature. Whether BAT or skeletal muscle activity is mediating the relationship between Personal-ET and WT has not been studied before.

OBJECTIVES: We examined whether BAT and skeletal muscles have a mediating role between Personal-ET and WT (as a proxy of peripheral vasoconstriction/vasodilation).

MATERIAL & METHODS: We quantified the levels of BAT by cold-induced ^{18}F-FDG-PET/CT scan, and the Personal-ET and WT by iButtons, in 75 participants (74% women).

RESULTS: We found that BAT volume and metabolic activity play a positive and significant role (up to 25.4%) in the association between Personal-ET and WT. In addition, we found that at the coldest temperatures, the participants with lower levels of WT (inducing higher peripheral vasoconstriction) had higher levels of BAT-outcomes, whereas in warmes temperatures we found that participants with higher levels of WT (inducing higher peripheral vasodilation) had lower levels of BAT-outcomes. We did not find any mediating role of skeletal muscle activity.

CONCLUSION: BAT volume and metabolic activity play a role in the relationship between Personal-ET and WT. Moreover, the data suggest that there are two distinct phenotypes: individuals who respond better to cold, both through non-shivering thermogenesis and peripheral vasoconstriction, and individuals who respond better to hot.

77 **Keywords:** Brown fat, thermoregulation, skin temperature, Temperatus®.

INTRODUCTION

The regulation of core body temperature is one of the most critical functions of the human body (39). Core body temperature is regulated by behavioral and physiological mechanisms (3, 39). Behavioral strategies are voluntary and oriented responses that help to maintain core body temperature, such as modifying posture, wearing clothing in winter, or using cold-air-conditioning in summer (3). On the other hand, physiological mechanisms are involuntary responses that generate or dissipate heat. In mammals, four physiological mechanisms are particularly involved in thermoregulation (39): (i) water evaporation (sweating), (ii) control of the skin blood flow, (iii) non-shivering thermogenesis (NST), and (iv) shivering thermogenesis. These mechanisms constantly interact, and their main aim is to keep the core body temperature in a normal range.

Skin temperature is a feedforward mechanism of the thermoregulatory system (39). When a change in the ambient temperature is detected by skin thermoreceptors, these trigger thermoregulatory responses that prevent any change in core body temperature (34). When humans are exposed to warm environments, peripheral blood vessels are dilated in order to promote heat loss (vasodilation), whereas in cold environments, peripheral blood vessels are constricted to prevent heat loss (vasoconstriction) (39). In animals, the engagement of specific thermoregulatory strategies is hierarchical (38). For instance, vasoconstriction occurs before NST, because vasoconstriction energy efficiency is higher than NST activation at least in mice models (26, 38). However, whether skin blood flow regulation mechanisms work hierarchically or concomitantly with NST activation or inhibition has not yet been studied in humans.

Both brown adipose tissue (BAT) and some skeletal muscles (40) play a role in NST. BAT is a specialized tissue for the rapid production of heat when the body is exposed to cold temperatures, which is mediated by the action of the uncoupling protein 1 (6). In

humans, BAT is mainly metabolically active upon cold exposure (9, 19, 41). However, BAT consume large quantities of energy expenditure in small mammals, although its contribution to NST in humans seems to be negligible, being the skeletal muscle the main effector of NST (5, 29, 40) and shivering (muscle contractions) during cold exposure (11). However, the contribution of BAT and skeletal muscle in the regulation of thermogenesis is largely unknown (1, 28, 40).

There are several ways to assess environmental temperature exposure (21, 31). Some studies quantified the personal level of environmental temperature (Personal-ET) (21), measured by an iButton during a period of 7 days. This iButton is always with the participant and should be in direct contact with the air (never with the skin) (25). This is thus a surrogate marker of temperature exposure of every individual. Other studies (4, 20) quantified a proxy of skin blood flow mechanisms (16, 35) and chronobiology (37) outcomes attaching an iButton to the wrist, measuring the wrist temperature (WT), normally at the same time that the Personal-ET. Personal-ET is related to WT (21); however, since cold and warm exposures have a direct effect on activation or inhibition of BAT and skeletal muscle, it could be that these thermogenic tissues might have a mediating role between Personal-ET and WT.

Based on the aforementioned, we studied the mediating role of BAT and skeletal muscle activity [assessed by cold-induced ^{18}F-Fluorodeoxyglucose (^{18}F-FDG) uptake] between Personal-ET and WT in young healthy adults for 7 days (24 hours/day). In order to understand the physiological mechanisms, we examined whether the association of the number of hours exposed to a certain Personal-ET with BAT and skeletal muscle ^{18}F-FDG uptake is mediated by WT as a surrogate marker of skin blood flow mechanisms.

MATERIAL & METHODS

A total of 90 (n=65 women) white Caucasian healthy adults aged 21.9 ± 2.3 years old participated in the present study (Table 1). The participants were enrolled in the ACTIBATE study (36), an exercise-based randomized controlled trial (Clinical Trials.gov ID: NCT02365129). All participants were non-smokers, were not enrolled in a weight loss program, had a stable body weight (body weight changes <3 kg) over the previous 3 months, were not physically active (<20 minutes on <3 days/week), did not take any medication, had no acute or chronic illness, and reported not to be regularly exposed to cold. The study was conducted in Granada (Southern Spain) between October and November in 2015 and 2016. The study protocol and informed consent were conducted in accordance with the Declaration of Helsinki (revision of 2013), and they were approved by the Human Research Ethics Committee of both the University of Granada (nº 924) and the Servicio Andaluz de Salud (Centro de Granada, CEI-Granada). A written informed consent was obtained from all the participants.

Wrist and Personal Environmental temperatures measurements

All participants wore 2 iButtons (DS-1922 L, Thermochron; resolution: 0.0625 °C; frequency: 10 min intervals; Maxim, Dallas, USA) for 7 days. One iButton was placed on the ventral side of the wrist of the non-dominant hand over the radial artery with a wrist band in order to determine WT. We instructed the participants to wear the iButton on the wrist for the whole day (even when asleep) and to take it off only when bathing or swimming. A second iButton was attached to a plastic fob and was used to quantify the Personal-ET. This iButton remained with the participant at all times but was never in direct contact with the body (21) or under clothing. During sleep-phases, the Personal-ET sensor was placed on the bedside table. The iButtons were programmed to start the recording at 06.00 and to finish 7 days later at 12.00 in the morning when the

152 ^{18}F-FDG positron emission tomography in combination with a computed tomography
153 scan (^{18}F-FDG) positron emission tomography with computed tomography (PET/CT)
154 scan was performed. The participants registered the non-wear periods in a diary during
155 the 7 days. We excluded the non-wear periods as well as those participants with less
156 than 5 valid days. For a day to be considered valid at least 75% of the day had to be
157 registered (≥18 hours). All iButtons were programmed and analyzed with the
158 Temperatus® software (http://profith.ugr.es/temperatus?lang=en). We calculated an
159 average of the valid recordings for the 7 days for both WT and Personal-ET separately.
160 Moreover, we calculated the number of hours per day that the participants were exposed
161 to a certain temperature with a 1ºC-range from 11 to 42ºC for the Personal-ET (e.g. 11-
162 11.99ºC, 12-12.99ºC, etc.) and from 29 to 37ºC for WT (e.g. 29-29.99ºC, 30-30.99ºC,
163 etc.).

164 *Personalized cooling protocol*

165 The personalized cooling protocol has been explained in detail elsewhere (24). Briefly,
166 the participants entered a mild-cold room (around 19.5ºC), and they were asked to wear
167 a water perfused cooling vest (Polar Products Inc., Ohio, USA). We determined the
168 participant's shivering threshold, reducing the water temperature gradually until
169 shivering occurred. Shivering was determined both visually by researchers as well as
170 self-reported by the participants. After 48-72 hours, we exposed the participants to 2
171 hours at their personalized temperature to induce maximum non shivering
172 thermogenesis (above ~4ºC) (17). After 1 hour of cold exposure, we injected a bolus of
173 ^{18}F-FDG (~185MBq), and we increased the water temperature 1ºC in order to prevent
174 shivering. After 2 hours of cold exposure we performed the PET/CT scan from the atlas
175 vertebrae to the thoracic vertebra 6. The evaluations were performed in 4 different
176 weeks among 2 months (from October to November 2016) in Granada, Spain.

177 *Quantification of ^{18}F-FDG uptake by BAT and skeletal muscle*

178 We quantified BAT volume and activity following the recently published
179 recommendations (7). PET/CT images were analyzed using the Beth Israel plugin for
180 FIJI (24) software by BMT with the supervision of a nuclear medicine physician. We
181 applied an individualized standardized uptake value (SUV) threshold [1.2/(lean body
182 mass/body mass)] (7) with a fixed range of Hounsfield units (HU, −190 to −10). We
183 quantified BAT volume and activity (i.e. SUV_{mean}, SUV_{peak}). We computed BAT
184 metabolic activity as BAT volume*SUV_{mean} (24) as well as the ^{18}F-FDG uptake by a
185 reference tissue (descending aorta). We quantified the ^{18}F-FDG uptake (SUV_{peak}) of
186 several skeletal muscles between the atlas vertebrae and the thoracic vertebra 4. We
187 drew a single region of interest (ROI) from 1 slice in paracervical, sternocleidomastoid,
188 scalene, longus colli, trapezius, parathoracic, supraspinatus, subscapular, deltoid,
189 pectoralis major, and triceps brachii muscles from both left and right sides of the body
190 (5, 13). An average of both sides including all skeletal muscles was calculated in order
191 to obtain a single representative value of the skeletal muscle glucose uptake of the upper
192 part of the body. Our protocol has shown a high inter-observer reliability, regardless of
193 the threshold applied to quantify BAT (23).

194 *Body composition*

195 Body composition was assessed on a separate day by Dual Energy X-ray
196 Absorptiometry (HOLOGIC, Discovery Wi) (36). The participants' weight and height
197 were measured without shoes and wearing a T-shirt and shorts using a SECA scale and
198 stadiometer (model 799, Electronic Column Scale, Hamburg, Germany), and we
199 calculated body mass index (BMI) (kg/m^2).

200 *Statistical analysis*

The descriptive characteristics of the study sample are presented as mean and standard deviation (SD) unless otherwise stated. There was no sex interaction (all P>0.10) in any of the study variables, thus we conducted the analyses in men and women together.

To quantify the mediating role of BAT volume, activity (i.e. SUV_{mean}, SUV_{peak}), and metabolic activity, and skeletal muscle activity in the relationship between Personal-ET and WT, we conducted mediation analyses (15). In addition, we tested the mediating role of WT on the association of the number of hours per day exposed to a certain Personal-ET with BAT volume and activity, and skeletal muscle activity. We used the PROCESS macro version 3.0, model four, with 5.000 bias-corrected bootstrap samples and 95% confidence intervals. Bootstrapping is a nonparametric resampling procedure which does not require the assumption of normality of the sampling distribution (33). The mediation was estimated using the indirect effect, which indicates the change on the effect of the independent variable on the outcome that can be endorsed to the proposed mediator. Indirect effects (*a*b* paths) with confidence intervals not including zero are interpreted as statistically significant (14), which could occur regardless of the significance of the total effect (c path, effect of the independent variable on the dependent variable) and the direct effect (c' path, effect on the dependent variable when both the independent and the mediator variables are included as independent variables) (15). To quantify how much of the total effect was due to the mediation, we calculated the percentage of mediation [(indirect effect / total effect) x 100], provided when the total effect was larger than the indirect effect with the same direction (15). All the analyses were performed using the IBM SPSS Statistics for Windows version 22.0 (Armonk, NY: IBM Corp), and the level of significance was set at P<0.05.

RESULTS

Table 1 shows the characteristics of the participants. A total of 15 out of 90 participants were excluded because less than 5 valid days of temperature had been recorded. A total of 75 participants (74.6% women) were finally included in the analyses, with 6.3 ± 0.5 valid days. The average age was 21.9 ± 2.3 years old and with a BMI of 25.2 ± 4.8 kg/m^2.

The mediating role of BAT

Figure 1 shows the mediating effect of BAT volume, activity (i.e. SUV_{mean} and SUV_{peak}), BAT metabolic activity, and skeletal muscle activity (SUV_{peak}) in the relationship between Personal-ET and WT. Personal-ET was positively associated with WT (*c* path= 0.0763; P=0.0014) and negatively associated with BAT-related outcomes (volume, SUV_{mean}, SUV_{peak}, and metabolic activity, *a* path, all P<0.001, see Figure 1 panels A, B, C, D, respectively) and skeletal muscle activity (*a* path, P=0.0023, see Figure 1E). BAT-related outcomes and skeletal muscle activity were not significantly associated with WT (*b* path). After including the mediator variables in the model (see Figure 1 *c´* path; all P<0.05), the direct effect of Personal-ET on WT remained statistically significant. The percentages of mediation of BAT volume and metabolic activity in the relationship between Personal-ET and WT were 25.4% and 23.9%, respectively. However, we did not observe any mediating effect of BAT activity (i.e., SUV_{mean} and SUV_{peak}) and skeletal muscle activity in the relationship between Personal-ET and WT (see Figure 1 panels: B, C, and E, respectively). These results persisted after controlling for sex, BMI, FMI, or LMI (data not shown). Furthermore, we repeated the analyses using BAT-related outcomes as well as skeletal muscle activity multiplied by lean body mass percentage (18) and the results remained unchanged (data not shown).

The mediating role of WT

Figure 2A shows the mediating effect of WT in the relationship between the number of hours exposed to a certain Personal-ET and BAT-related outcomes (volume, SUV_{peak}, and metabolic activity). The number of hours per day exposed to a warm Personal-ET was negatively associated with BAT volume (from 25ºC to 28ºC; *c* path; all P<0.05) and positively associated with WT (from 24ºC to 27ºC; *a* path; all P<0.05) (Table S1). WT was also negatively associated with BAT volume at this temperature range (*b* path; all P<0.05). The direct effect was only significant when examining the number of hours per day exposed to temperatures ≥26ºC (*c'* path; all P<0.05) (Table S1). WT showed the highest percentage of mediation (57%) in the relationship between the number of hours exposed to 24ºC and BAT volume in comparison with other ranges of warm temperatures (see Figure 2E). In addition, we observed that the number of hours per day exposed to a cold Personal-ET was positively related to BAT volume (from 14ºC to 20ºC; c path; all P<0.05) and negatively associated with WT (from 16ºC to 20ºC; a path; all P<0.05) (Table 2S). WT was negatively associated with BAT volume (b path; P<0.05) and the association between the number of hours exposed to a cold temperature (from 16ºC to 19ºC) and BAT volume persisted after including WT as a mediator (c' path; both; all P<0.05). The sign of the indirect effect changed during the ambient exposure, being positive during cold-ambient exposure and negative during warm-ambient exposures (see Figure 2B-G). Moreover, when the participants were exposed to a certain range of temperature in the thermoneutral zone, WT did not play a mediating role in BAT volume (from 21ºC to 23ºC, see Figures 2B and E). The mediation analyses were performed for the number of hours exposed to each degree of Personal-ET showing that the mediating effect disappeared at temperatures ≥28ºC or ≤14ºC, probably due to a lack of statistical power at these ranges (small number of participants

276 exposed to these extreme temperatures). The mediating role of WT was also observed in
277 the relationship between Personal-ET and BAT activity (i.e. SUV_{peak} and metabolic
278 activity, see Figure 2 for the indirect effect: panels C and D, respectively, and for the
279 percentage of mediation: panels F and G, respectively; see Table S2 and S3 for further
280 details). Furthermore, we did not find a mediating effect of the WT on the association of
281 the number of hours exposed to a certain Personal-ET with SUV_{mean} and skeletal muscle
282 activity (data not shown), as well as in upper (>29ºC) and lower (<13ºC) ranges of
283 temperature due to the lack of statistical power in these ranges (data not shown). The
284 results persisted after controlling by sex, BMI, LMI, FMI, or date when the evaluation
285 were performed (data not shown). Overall, the results persisted, when we repeated all
286 the analyses excluding data regarding the temperature ranges, for both WT and
287 Personal-ET, when the participants were asleep (data not shown). Moreover, we
288 repeated the analyses using other classifications of skeletal muscles (5) activity
289 (SUV_{peak}) and the absence of a mediating role of this tissue persisted (data not shown).

290 **DISCUSSION**

291 The present study quantifies, for the first time, the mediating role of human BAT and
292 skeletal muscle cold-induced activity in the relationship between personal level of
293 environmental temperature and human wrist temperature as an indirect proxy of skin
294 blood flow. Intriguingly, the results show that BAT volume and metabolic activity
295 mediate up to 25.4% of the association between Personal-ET and WT. Moreover, the
296 results indicate that the association of the number of hours exposed to a certain
297 Personal-ET with BAT volume, SUV_{peak}, and metabolic activity is mediated by WT at
298 temperatures from 14ºC to 20ºC and from 24ºC to 28ºC, but not in the thermoneutral
299 zone, as expected. We did not find a mediating role of human skeletal muscles or a
300 relationship between WT and skeletal muscles. We also found that the participants with

lower WT (inducing higher peripheral vasoconstriction) at the coldest temperatures had higher levels of BAT volume, SUV_{peak} and metabolic activity, whereas the participants with higher WT (inducing higher peripheral vasodilation) at the warmest temperatures had lower levels of BAT volume, SUV_{peak} and metabolic activity. These findings show how WT (as a proxy of blood flow) is related to BAT volume and activity (SUV_{peak}) in young adults. However, further studies are needed to elucidate the possible mechanisms behind these relationships.

The mediating role of BAT

We show that both BAT volume and metabolic activity have a mediating role in the relationship between Personal-ET and WT measured in daily living conditions independently of the sex, BMI, LMI and FMI. This indicates that participants with who were exposed to the same Personal-ET during the 7 day had different WT, which is explained, at least in part, by different levels of BAT volume or metabolic activity. Therefore, by every 1ºC that the personal-ET is decreased, BAT volume would explain approximately an increase of 0.0194ºC in WT. The relationship between Personal-ET and the WT daily pattern has been widely used in the field of chronobiology (21, 22). Several studies compared WT daily patterns in obese vs. normal-weight women (8), young vs. older men and women (4, 16), and men vs. women (20), and showed worse patterns (higher variability and higher daytime values) of WT in obese and older participants. These findings are also in accordance with those of human BAT studies, which showed that obese, older people, and men have lower BAT volume and activity (32). Therefore, we postulate that BAT volume and metabolic activity should be taken into account in further chronobiological studies using WT, especially in those studies, which only measured WT as a proxy of the circadian pattern without the inclusion of the Personal-ET. We established that based on the following facts: (i) the observed

mediating role of human BAT volume (and metabolic activity) in the relationship between Personal-ET and WT, (ii) the activation of BAT in cold ambient-temperatures (Personal-ET≤20ºC), (iii) that obese, older people, and men have lower BAT volume and activity as well as worse patterns of WT, and (iv) the circadian rhythms and, specifically core body temperature rhythms are all controlled by specific neural pathways in the anterior hypothalamus (39). For instance, Martinez-Nicolas et al. (21) studied the mediation role of WT in the relationship between Personal-ET and mean arterial blood pressure in summer and winter, and postulated that BAT could mediate this relationship. In this study, we show that this hypothesis might be true, although further studies are needed to fully understand the possible mechanisms behind these assumptions.

The mediating role of WT

All the physiological mechanisms of the thermoregulatory system seem to be orchestrated in the preoptic area (POA) of the hypothalamus (39). In addition to the peripheral tissues, the temperature of the brain is an input into the thermoregulatory system (12). One of the hypotheses explaining why human BAT is placed in the cervical and supraclavicular zone is because, as a thermogenic tissue, its main function is to regulate the temperature of the blood going to the brain (2, 42). Several studies have shown that human BAT activation is related to an increase in the blood flow in BAT (28, 30). Based on these results, the present study postulate that the increase in BAT activation (blood flow) could result in a redistribution of the blood in the peripheral part of the body during a cold stimulus which is moved into BAT in order to generate heat, since BAT is highly irrigated (27). In contrast, during a warm ambient, the blood flow in the peripheral part of the body increases at the same time as BAT blood flow and activation decrease.

Warm

In warm-ambient environments (from 24ºC to 28ºC), we observed that the higher the Personal-ET is, the higher WT is, which is associated with a lower BAT volume and activity. Therefore, by every hour exposed at 27ºC (personal-ET), WT would increase and explain approximately a decrease of 3.2 ml of BAT volume. The skin has warm-sensitive neurons specially to perceive the warm exposures (39). However, there is some controversy as to which the main transient receptor potential (TRP) channel to be involved as a warm sensor is, the candidates being TRPV1, TPRV3, TPRV4, and TRPM2 (39). Therefore, there might be participants with higher or more number of TRP channels than others, and this fact could explain why there are different responses to the same stimulus, although further studies are needed. Regardless of the main TRP channel involved, our results suggest that when Personal-ET is high (hot), the body initiates some physiological response in order to preserve core temperature. Thus, the main physiological mechanism involved is to induce a peripheral vasodilation with an inhibition of human BAT (redistribution of blood flow to peripheral regions to dissipate heat). We also showed that the higher the WT is, the lower the levels of BAT volume and activity (inhibition of this tissue) are. For instance, two participants that spent the same time in warm ambient, the participant with higher WT also had lower levels of BAT volume, which might indicate more efficiency adapting to warm temperatures, which reciprocally would implicated less efficient response to cold.

Cold

In cold-ambient exposures (from 14ºC to 19ºC), we showed that the lower the Personal-ET is, the lower WT is, which is associated with higher BAT volume and activity. Therefore, by every hour exposed at 15ºC (personal-ET), WT would decrease and explain approximately an increase of 2.5 ml of BAT volume. In the skin of the

peripheral parts of the body, there are also cold-sensitive neurons. These cold-sensitive neurons highly expressed levels of transient receptor potential cation channel subfamily M member 8 (TRMP8), which is the primary peripheral cold sensor in the thermoregulatory system (10). Animal models have shown that the inhibition of this sensor inhibits the behavioral and physiological responses to cooling (10). Taking this into consideration, we can postulate that there are individuals with a more efficient thermoregulatory system against cold stimuli, inducing a higher peripheral vasoconstriction and BAT activation in order to keep the core body temperature constant, which could be explained by a higher sympathetic tone. According to this, it might be possible for people with higher levels of human BAT to have a higher concentration of TRMP8, as well as different polymorphism of the gene TRMP8 might be associated with a better response to cold stimuli; however, these hypotheses have not been studied so far.

The mediating role of skeletal muscles

Skeletal muscles are involved in the thermoregulatory responses during cold exposure (5, 40). Interestingly, we did not observe an effect of the skeletal muscle activity (as measured by the ^{18}F-FDG uptake) in the relationship between Personal-ET and WT. This lack of mediating effect does not necessarily mean that skeletal muscle is not involved in cold-induced thermogenesis. This lack of effect might be due to the fact that the cold-ambient temperatures were not cold enough to induce skeletal muscle activation, or because the ^{18}F-FDG tracer is not a good marker of skeletal muscle metabolism (40).

We postulate, however, that there are participants who respond better (i.e. responders) than others to cold exposures, and others that respond better to warm exposures, yet further studies are needed. This assumption is also based on the fact that some people

401 could have an overexpression of POA neuron levels or TRMP8 or TRP channels (39),
402 making the thermoregulatory system more efficient, or maybe in the brain the areas
403 involved in the thermoregulatory system are different. This cross-sectional and
404 observational study should be replicated in older participants and using other nuclear
405 tracers such as ^{15}O-O_2, [^{11}C]-acetate (40), or adenosine perfusion, a vasodilator that
406 seems to active human BAT (23). Moreover, we know that during sleep phases humans
407 can lose at least 25% of their total thermoregulatory capacity. Since our aim was to
408 study the mediating role of human BAT during 7 days (even in sleep phases) we keep
409 these analyses as main results, although excluding the sleep phase did not alter the
410 results (data not shown). Moreover, in this study the level of clothing during the
411 measurements were not evaluated. Future experimental studies are warranted to
412 elucidate the possible mechanisms behind this efficiency in the thermoregulatory
413 system and new therapies that could be developed to improve this physiological system.

414 **CONCLUSIONS**

415 We show that BAT volume and metabolic activity mediate the relationship between
416 Personal-ET and WT. Moreover, our data support that the individuals who were
417 exposed to lower environmental temperatures and at the same time had lower wrist skin
418 temperature, concomitantly had higher BAT volume. We also observed the opposite
419 effect when the participants were exposed to warmer temperatures, which indicates a
420 redistribution of the blood flow between the peripheral part of the body and BAT
421 activation/inhibition in order to keep the core body temperature constant. Future
422 interventional studies should try to find strategies to improve the thermoregulatory
423 system and its relationship with metabolic diseases.

424

AUTHOR CONTRIBUTIONS

Conception and design of research: B.M.T. and J.R.R.; B.M.T., F.A.M, G.S.D., and J.M.L.E. performed the experiments; M.A.R., B.M.T., V.M.V., and J.R.R. analyzed the data; All authors interpreted the results; M.A.R. and B.M.T. prepared the figures and drafted manuscript; All authors critically revised the manuscript and approved the final version.

REFERENCES

1. **Acosta FM**, **Martinez-tellez B**, **Sanchez-delgado G**, **Juan MA**, **Acosta-manzano P**, **Morales-artacho AJ**, **Ruiz JR**. Physiological responses to acute cold exposure in young lean men. (2018). doi: 10.6084/m9.figshare.6182180.

2. **Bahler L**, **Holleman F**, **Booij J**, **Hoekstra JB**, **Verberne HJ**. Hot heads & cool bodies: The conundrums of human BAT activity research. *Eur. J. Intern. Med.* (January 5, 2017). doi: 10.1016/j.ejim.2016.12.023.

3. **Batchelder P**, **Kinney RO**, **Demlow L**, **Lynch CB**. Effects of temperature and social interactions on huddling behavior in Mus musculus. *Physiol Behav* 31: 97–102, 1983.

4. **Batinga H**. Ontogeny and aging of the distal skin temperature rhythm in humans. (2015). doi: 10.1007/s11357-015-9768-y.

5. **Blondin DP**, **Labbé SM**, **Phoenix S**, **Guérin B**, **Turcotte ÉE**, **Richard D**, **Carpentier AC**, **Haman F**. Contributions of white and brown adipose tissues and skeletal muscles to acute cold-induced metabolic responses in healthy men. *J Physiol* 593: 701–14, 2015.

6. **Cannon B**, **Nedergaard J**. Brown adipose tissue: function and physiological

significance. *Physiol Rev* 84: 277–359, 2004.

7. **Chen KY**, **Cypess AM**, **Laughlin MR**, **Haft CR**, **Hu HH**, **Bredella MA**, **Enerbäck S**, **Kinahan PE**, **Lichtenbelt W van M**, **Lin FI**, **Sunderland JJ**, **Virtanen KA**, **Wahl RL**. Brown Adipose Reporting Criteria in Imaging STudies (BARCIST 1.0): Recommendations for Standardized FDG-PET/CT Experiments in Humans. *Cell Metab* 24: 210–222, 2016.

8. **Corbalán-Tutau MD**, **Madrid JA**, **Ordovás JM**, **Smith CE**, **Nicolás F**, **Garaulet M**. Differences in daily rhythms of wrist temperature between obese and normal-weight women: Associations with metabolic syndrome features. *Chronobiol Int* 28: 425–433, 2011.

9. **Cypess AM**, **Lehman S**, **Williams G**, **Tal I**, **Rodman D**, **Goldfine AB**, **Kuo FC**, **Palmer EL**, **Tseng Y-H**, **Doria A**, **Kolodny GM**, **Kahn CR**. Identification and importance of brown adipose tissue in adult humans. *N Engl J Med* 360: 1509–17, 2009.

10. **Dhaka A**, **Murray AN**, **Mathur J**, **Earley TJ**, **Petrus MJ**, **Patapoutian A**. TRPM8 Is Required for Cold Sensation in Mice. *Neuron* 54: 371–378, 2007.

11. **Haman F**, **Blondin DP**. Shivering thermogenesis in humans: origin, contribution and metabolic requirement. *Temperature* : 0–0, 2017.

12. **Hammel HT**, **Pierce JB**. Regulation of Internal Body Temperature. *Annu Rev Physiol* 30: 641–710, 1968.

13. **Hanssen MJW**, **van der Lans AAJJ**, **Brans B**, **Hoeks J**, **Jardon KMC**, **Schaart G**, **Mottaghy FM**, **Schrauwen P**, **van Marken Lichtenbelt WD**. Short-term Cold Acclimation Recruits Brown Adipose Tissue in Obese Humans.

Diabetes 65: 1179–89, 2016.

14. **Hayes AF**. Beyond Baron and Kenny : Statistical Mediation Analysis in the New Millennium Beyond Baron and Kenny : Statistical Mediation Analysis in the New Millennium. *Commun Monogr* 76: 408–420, 2009.

15. **Hayes AF**. *Introduction to mediation, moderation, and conditional process analysis : a regression-based approach*. Guildford Press, 2013.

16. **Kräuchi K**, **Gompper B**, **Hauenstein D**, **Flammer J**, **Pflüger M**, **Studerus E**, **Schötzau A**, **Orgül S**. Diurnal blood pressure variations are associated with changes in distalproximal skin temperature gradient. *Chronobiol Int* 29: 1273–1283, 2012.

17. **van der Lans a. a. JJ**, **Wierts R**, **Vosselman MJ**, **Schrauwen P**, **Brans B**, **van Marken Lichtenbelt WD**. Cold-activated brown adipose tissue in human adults: methodological issues. *AJP Regul Integr Comp Physiol* 307: R103–R113, 2014.

18. **Leitner BP**, **Huang S**, **Brychta RJ**, **Duckworth CJ**, **Baskin AS**, **McGehee S**, **Tal I**, **Dieckmann W**, **Gupta G**, **Kolodny GM**, **Pacak K**, **Herscovitch P**, **Cypess AM**, **Chen KY**. Mapping of human brown adipose tissue in lean and obese young men. *Proc Natl Acad Sci U S A* 114: 8649–8654, 2017.

19. **van Marken Lichtenbelt WD**, **Vanhommerig JW**, **Smulders NM**, **Drossaerts JM a FL**, **Kemerink GJ**, **Bouvy ND**, **Schrauwen P**, **Teule GJJ**. Cold-activated brown adipose tissue in healthy men. *N Engl J Med* 360: 1500–1508, 2009.

20. **Martinez-Nicolas A**, **Guaita M**, **Santamaría J**, **Montserrat JM**, **Rol MÁ**, **Madrid JA**. Circadian impairment of distal skin temperature rhythm in patients with sleep-disordered breathing: The effect of CPAP. *Sleep* 40: 31–37, 2017.

21. **Martinez-Nicolas A**, **Meyer M**, **Hunkler S**, **Madrid JA**, **Rol MA**, **Meyer AH**, **Schötzau A**, **Orgül S**, **Kräuchi K**. Daytime variation in ambient temperature affects skin temperatures and blood pressure: Ambulatory winter/summer comparison in healthy young women. *Physiol Behav* 149: 203–11, 2015.

22. **Martinez-Nicolas A**, **Ortiz-Tudela E**, **Rol MA**, **Madrid JA**. Uncovering Different Masking Factors on Wrist Skin Temperature Rhythm in Free-Living Subjects. *PLoS One* 8, 2013.

23. **Martinez-Tellez B**, **Nahon KJ**, **Sanchez-Delgado G**, **Abreu-Vieira G**, **Llamas-Elvira JM**, **van Velden FHP**, **Pereira Arias-Bouda LM**, **Rensen PCN**, **Boon MR**, **Ruiz JR**. The impact of using BARCIST 1.0 criteria on quantification of BAT volume and activity in three independent cohorts of adults. *Sci Rep* 8: 8567, 2018.

24. **Martinez-Tellez B**, **Sanchez-Delgado G**, **Garcia-Rivero Y**, **Alcantara JMA**, **Martinez-Avila WD**, **Muñoz-Hernandez M V.**, **Olza J**, **Boon MR**, **Rensen PCN**, **Llamas-Elvira JM**, **Ruiz JR**. A New Personalized Cooling Protocol to Activate Brown Adipose Tissue in Young Adults. *Front Physiol* 8: 1–10, 2017.

25. **Martinez-Tellez B**, **Xu H**, **Sánchez-Delgado G**, **Acosta FM**, **Rensen PCN**, **Llamas-Elvira JM**, **Ruiz JR**. Association of wrist and ambient temperature with cold-induced brown adipose tissue and skeletal muscle 18F-FDG uptake in young adults. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* (October 26, 2018). doi: 10.1152/ajpregu.00238.2018.

26. **McAllen RM**, **Tanaka M**, **Ootsuka Y**, **McKinley MJ**. Multiple thermoregulatory effectors with independent central controls. *Eur J Appl Physiol* 109: 27–33, 2010.

27. **Muzik O**, **Mangner TJ**, **Leonard WR**, **Kumar A**, **Granneman JG**. Sympathetic Innervation of Cold-Activated Brown and White Fat in Lean Young Adults. *J. Nucl. Med.* (2016). doi: 10.2967/jnumed.116.180992.

28. **Muzik O**, **Mangner TJ**, **Leonard WR**, **Kumar A**, **Janisse J**, **Granneman JG**. 15O PET measurement of blood flow and oxygen consumption in cold-activated human brown fat. *J Nucl Med* 54: 523–31, 2013.

29. **Muzik O**, **Mangner TJ**, **Leonard WR**, **Kumar A**, **Janisse J**, **Granneman JG**. 15O PET Measurement of Blood Flow and Oxygen Consumption in Cold-Activated Human Brown Fat. *J Nucl Med* 54: 523–531, 2013.

30. **Orava J**, **Nuutila P**, **Lidell ME**, **Oikonen V**, **Noponen T**, **Viljanen T**, **Scheinin M**, **Taittonen M**, **Niemi T**, **Enerbäck S**, **Virtanen K a**. Different metabolic responses of human brown adipose tissue to activation by cold and insulin. *Cell Metab* 14: 272–279, 2011.

31. **Ouellet V**, **Routhier-Labadie A**, **Bellemare W**, **Lakhal-Chaieb L**, **Turcotte E**, **Carpentier AC**, **Richard D**. Outdoor temperature, age, sex, body mass index, and diabetic status determine the prevalence, mass, and glucose-uptake activity of 18F-FDG-detected BAT in humans. *J Clin Endocrinol Metab* 96: 192–199, 2011.

32. **Ouellet V**, **Routhier-Labadie A**, **Bellemare W**, **Lakhal-Chaieb L**, **Turcotte E**, **Carpentier AC**, **Richard D**. Outdoor temperature, age, sex, body mass index, and diabetic status determine the prevalence, mass, and glucose-uptake activity of 18F-FDG-detected BAT in humans. *J Clin Endocrinol Metab* 96: 192–9, 2011.

33. **Preacher KJ**, **Hayes AF**. Asymptotic and resampling strategies for assessing and comparing indirect effects in multiple mediator models. *Behav Res Methods* 40: 879–891, 2008.

34. **Romanovsky AA**. Skin temperature: Its role in thermoregulation. *Acta Physiol* 210: 498–507, 2014.

35. **Rubinstein EH**, **Sessler DI**. Skin-surface temperature gradients correlate with fingertip blood flow in humans. [Online]. *Anesthesiology* 73: 541–5, 1990. http://www.ncbi.nlm.nih.gov/pubmed/2393139.

36. **Sanchez-Delgado G**, **Martinez-Tellez B**, **Olza J**, **Aguilera CM**, **Labayen I**, **Ortega FB**, **Chillon P**, **Fernandez-Reguera C**, **Alcantara JMA**, **Martinez-Avila WD**, **Muñoz-Hernandez V**, **Acosta FM**, **Prados-Ruiz J**, **Amaro-Gahete FJ**, **Hidalgo-Garcia L**, **Rodriguez L**, **Ruiz YA-K**, **Ramirez-Navarro A**, **Muros-de Fuentes MA**, **García-Rivero Y**, **Sanchez-Sanchez R**, **de Dios Beas Jimenez J**, **de Teresa C**, **Navarrete S**, **Lozano R**, **Brea-Gomez E**, **Rubio-Lopez J**, **Ruiz MR**, **Cano-Nieto A**, **Llamas-Elvira JM**, **Jimenez Rios JA**, **Gil A**, **Ruiz JR**. Activating brown adipose tissue through exercise (ACTIBATE) in young adults: Rationale, design and methodology. *Contemp Clin Trials* 45: 416–25, 2015.

37. **Sarabia JA**, **Rol MA**, **Mendiola P**, **Madrid JA**. Circadian rhythm of wrist temperature in normal-living subjects. A candidate of new index of the circadian system. *Physiol Behav* 95: 570–580, 2008.

38. **Satinoff E**. Neural organization and evolution of thermal regulation in mammals. [Online]. *Science* 201: 16–22, 1978. http://www.ncbi.nlm.nih.gov/pubmed/351802.

39. **Tan CL**, **Knight ZA**. Regulation of Body Temperature by the Nervous System. *Neuron* 98: 31–48, 2018.

40. **U Din M**, **Raiko J**, **Saari T**, **Kudomi N**, **Tolvanen T**, **Oikonen V**, **Teuho J**,

Sipilä HT, **Savisto N**, **Parkkola R**, **Nuutila P**, **Virtanen KA**. Human brown adipose tissue [(15)O]O2 PET imaging in the presence and absence of cold stimulus. *Eur. J. Nucl. Med. Mol. Imaging* (2016). doi: 10.1007/s00259-016-3364-y.

41. **Virtanen KA**, **Lidell ME**, **Orava J**, **Heglind M**, **Westergren R**, **Niemi T**, **Taittonen M**, **Laine J**, **Savisto N-J**, **Enerbäck S**, **Nuutila P**. Functional brown adipose tissue in healthy adults. *N Engl J Med* 360: 1518–25, 2009.

42. **Yoneshiro T**, **Matsushita M**, **Nakae S**, **Kameya T**, **Sugie H**, **Tanaka S**, **Saito M**. Brown adipose tissue is involved in the seasonal variation of cold-induced thermogenesis in humans. *Am J Physiol Regul Integr Comp Physiol* : ajpregu.00057.2015, 2016.

Table 1. Characteristics of the study participants

	n=75
Sex (% women)	74.6%
Age (years)	21.9 ± 2.3
Body mass index (kg/m^2)	25.2 ± 4.8
Lean mass (kg)	41.3 ± 9.6
Fat mass (kg)	26.9 ± 9.5
Fat mass (%)	37.6 ± 7.0
BAT volume (ml)	69.0 ± 61.3
BAT activity (SUV_{mean} g/ml)	3.7 ± 1.9
BAT activity (SUV_{peak} g/ml)	11.0 ± 8.5
Wrist Temperature (ºC)	34.0 ± 0.7
Personal-ET (ºC)	22.8 ± 3.1

Data are presented as mean and standard deviation, unless otherwise stated. BAT: brown adipose tissue, Personal-ET: Personal level of environmental temperature, SUV: standardized uptake value.

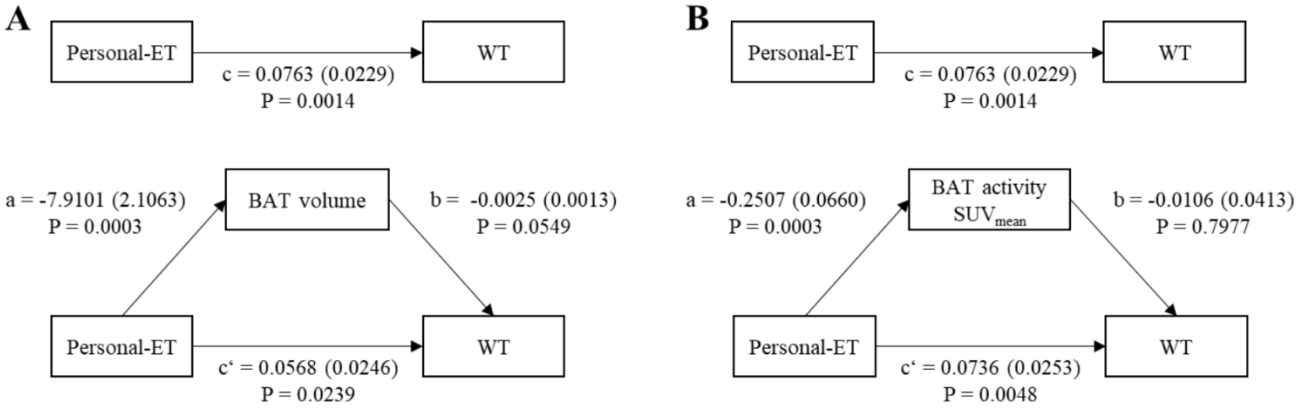
Figure 1. Mediation models of the relationship between personal levels of environmental temperature and wrist skin temperature with (A) BAT volume (ml), (B) SUV_{mean} (g/ml), (C) SUV_{peak} (g/ml), (D) metabolic activity (calculated as BAT volume x BAT SUV_{mean}), and (E) skeletal muscle activity (g/ml) included as mediator variables, respectively.

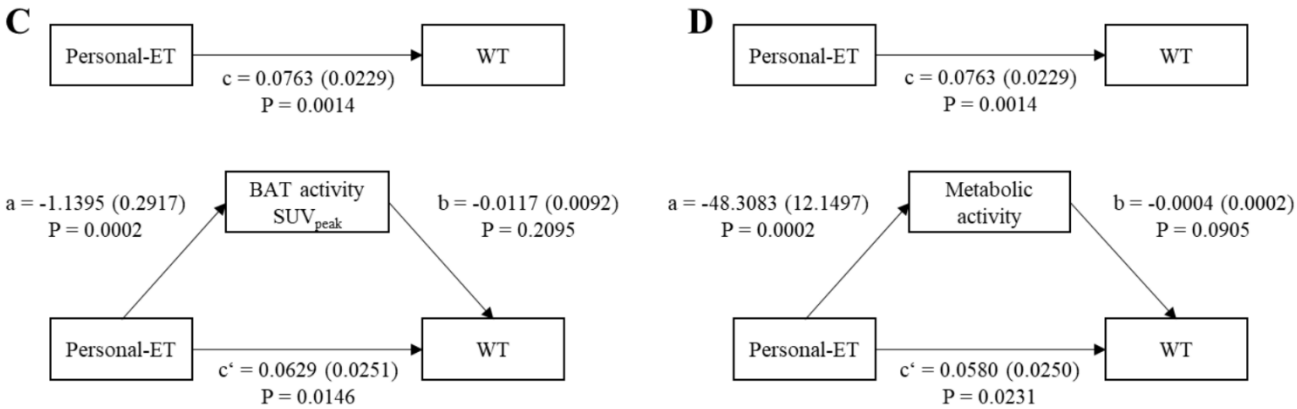
Paths a, b, c, and c' are presented as unstandardized coefficients (Standard Error, SE). β = indirect effect (*a*b* paths); [lower limit confident interval: upper limit confident interval], lower and upper levels for 95% confidence interval of the indirect effect based on 5000 bootstraps.

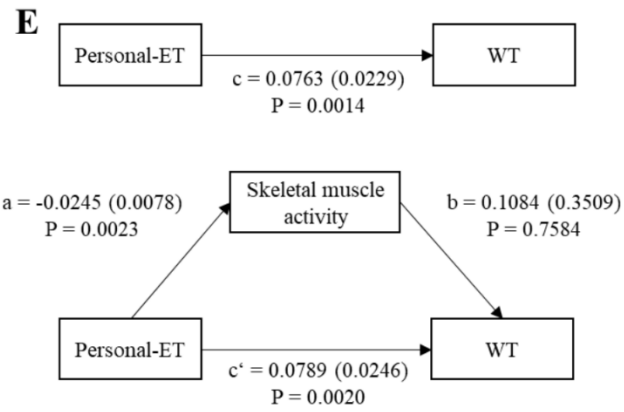
The results are shown as unstandardized coefficients (Standard Error, SE) and bias corrected 95% CI based on 5000 bootstraps.

Personal ET: personal levels of environmental temperature; WT: wrist skin temperature; BAT: Brown adipose tissue; P_M: percentage of mediation; SUV: Standardized uptake value: WT: wrist temperature.

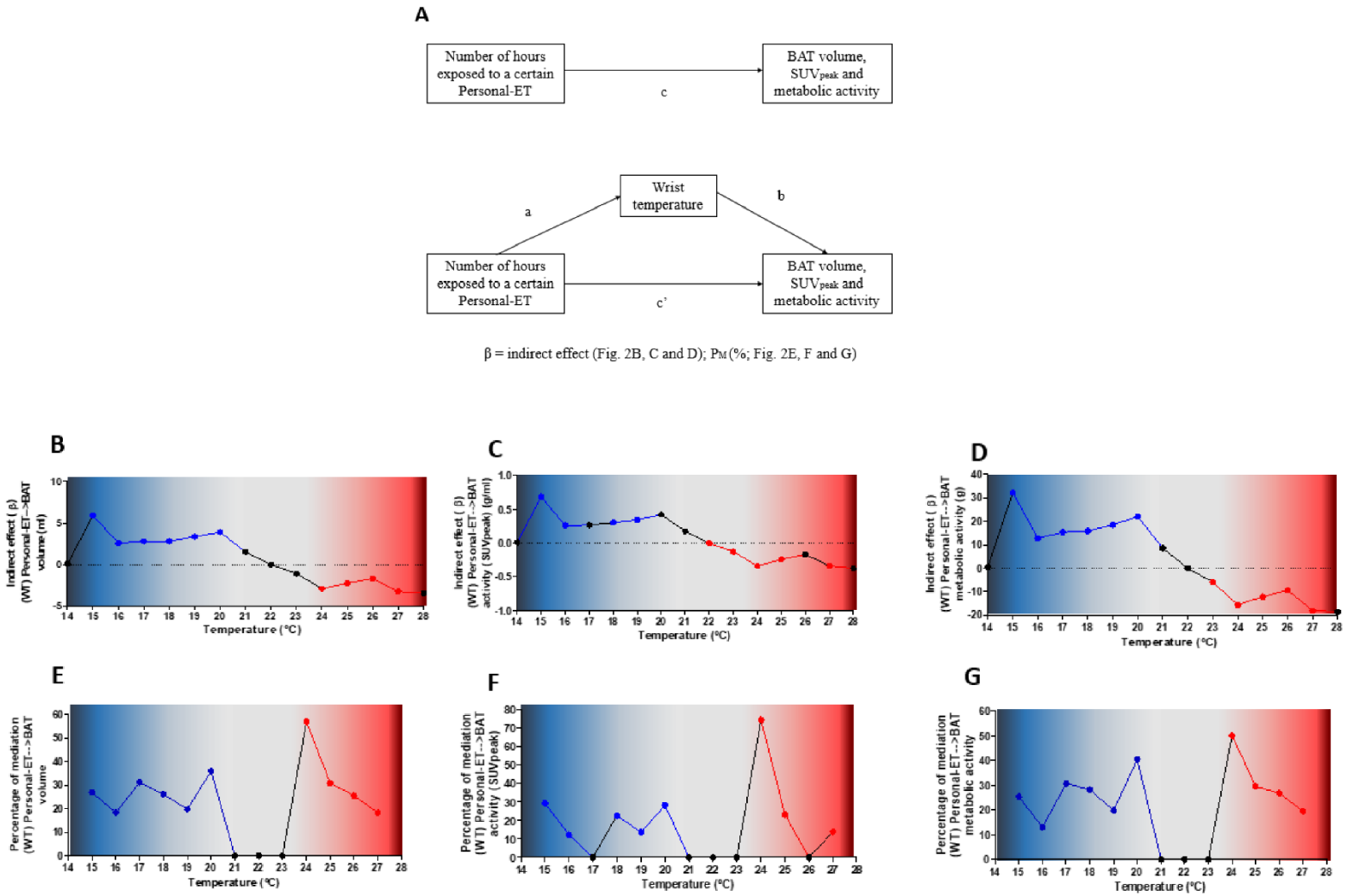
ns: non-significant.


A
Personal-ET
WT
c = 0.0763 (0.0229)
P = 0.0014
a = -7.9101 (2.1063)
P = 0.0003
BAT volume
b = -0.0025 (0.0013)
P = 0.0549
Personal-ET
WT
c‘ = 0.0568 (0.0246)
P = 0.0239
β = 0.0194 [0.0023; 0.0504]; P_M = 25.4%
B
Personal-ET
WT
c = 0.0763 (0.0229)
P = 0.0014
a = -0.2507 (0.0660)
P = 0.0003
BAT activity
SUV_mean
b = -0.0106 (0.0413)
P = 0.7977
Personal-ET
WT
c‘ = 0.0736 (0.0253)
P = 0.0048
β = 0.0027 [-0.0204; 0.0230]; ns


C
Personal-ET
WT
c = 0.0763 (0.0229)
P = 0.0014
a = -1.1395 (0.2917)
P = 0.0002
BAT activity
SUV_peak
b = -0.0117 (0.0092)
P = 0.2095
Personal-ET
WT
c‘ = 0.0629 (0.0251)
P = 0.0146
β = 0.0133 [-0.0056; 0.0558]; ns
D
Personal-ET
WT
c = 0.0763 (0.0229)
P = 0.0014
a = -48.3083 (12.1497)
P = 0.0002
Metabolic
activity
b = -0.0004 (0.0002)
P = 0.0905
Personal-ET
WT
c‘ = 0.0580 (0.0250)
P = 0.0231
β = 0.0182 [0.0014; 0.0463]; P_M = 23.9%


E
Personal-ET
WT
c = 0.0763 (0.0229)
P = 0.0014
a = -0.0245 (0.0078)
P = 0.0023
Skeletal muscle
activity
b = 0.1084 (0.3509)
P = 0.7584
Personal-ET
WT
c‘ = 0.0789 (0.0246)
P = 0.0020
β = -0.0027 [-0.0186; 0.0136]; ns

606 **Figure 2.** A) Mediation models of the relationship between the number of hours exposed to a certain
607 Personal-ET and BAT-related outcomes in young adults. Path c shows the association between
608 independent and dependent variables. Arrows a x b show the natural indirect effect (β) pathway, and c'
609 shows the natural direct effect pathway. B) Indirect effects (β) of the simple mediation analyses of wrist
610 temperature on the association between the number of hours exposed to each degree of Personal-ET (from
611 14ºC to 28ºC) and BAT volume, whereas panels C and D refer to BAT SUV_{peak} and metabolic activity,
612 respectively. E) P_M of the simple mediation analyses of wrist temperature on the association between the
613 number of hours exposed to each degree of personal-ET (from 14ºC to 28ºC) and BAT volume, whereas
614 panels F and G refer to BAT SUV_{peak} and metabolic activity, respectively. Black dots represent that 0 was
615 in the 95% confidence interval of the indirect effect, and, therefore, the mediation was considered non-
616 statistically significant (P>0.05). Red and blue dots mean that the mediation analysis was statistically
617 significant but with a different direction. BAT: brown adipose tissue; Personal-ET: personal level of
618 environmental temperature; WT: wrist skin temperature; P_M: Percentage of mediation.
619


A
Number of hours exposed to a certain Personal-ET
c
BAT volume, SUVpeak and metabolic activity
Wrist temperature
a
b
Number of hours exposed to a certain Personal-ET
c'
BAT volume, SUVpeak and metabolic activity
β = indirect effect (Fig. 2B, C and D); PM (%; Fig. 2E, F and G)
B
Indirect effect (β) (WT) Personal-ET-->BAT volume (ml)
Temperature (°C)
C
Indirect effect (β) (WT) Personal-ET-->BAT activity (SUVpeak) (g/ml)
Temperature (°C)
D
Indirect effect (β) (WT) Personal-ET-->BAT metabolic activity (g)
Temperature (°C)
E
Percentage of mediation (WT) Personal-ET-->BAT volume
Temperature (°C)
F
Percentage of mediation (WT) Personal-ET-->BAT activity (SUVpeak)
Temperature (°C)
G
Percentage of mediation (WT) Personal-ET-->BAT metabolic activity
Temperature (°C)

620

Table S1. Total, direct, and indirect effects of the simple mediation analyses investigating the mediating role of wrist temperature in the association between the number of hours exposed to a certain personal environmental temperature and brown adipose tissue volume.

Independent variable	Total effect (*c*)	Direct effect (*c'*)	Path *a*	Path *b*	Indirect effect (*a*b*)	BC 95% CI Lower Upper	P_M (%)
Number of hours per day exposed to							
28ºC	-24.1511 (8.5478)**	-20.7341 (8.2251)*	0.1172 (0.0952)	-29.1556 (10.0126)**	-3.4170	-13.6843; 2.2662	
27ºC	-17.5167 (4.8821)***	-14.2999 (4.9035)**	0.1296 (0.0545)*	-24.8155 (10.1490)*	**-3.2168**	-8.9958; -0.5952	18.36
26ºC	-6.5085 (1.9827)**	-4.8443 (2.0725)*	0.0702 (0.0211)**	-23.7003 (10.6935)*	**-1.6642**	-3.4272; -0.3454	25.57
25ºC	-7.2114 (2.8879)*	-4.9839 (2.9130)	0.0814 (0.0307)**	-27.3753 (10.6111)*	**-2.2275**	-5.4156; -0.4822	30.89
24ºC	-5.0423 (3.6912)	-2.1706 (3.6547)	0.0925 (0.0384)*	-31.0297 (10.7150)**	**-2.8717**	-5.8755; -0.9852	56.95
23ºC	-0.1435 (2.5060)	0.9153 (2.3847)	0.0318 (0.0265)	-33.3129 (10.4289)**	-1.0588	-2.6913; 0.0416	
22ºC	-1.7610 (2.8817)	-1.7698 (2.7173)	-0.0003 (0.0309)	-32.7633 (10.3079)**	0.0087	-1.6455; 2.1567	
21ºC	1.5069 (3.5844)	-0.0470 (3.4213)	-0.0474 (0.0379)	-32.7773 (10.4483)**	1.5539	-0.6785; 4.8597	
20ºC	10.8615 (5.0206)*	6.9503 (5.0396)	-0.1373 (0.0529)*	-28.4826 (10.6645)**	**3.9112**	1.1979; 9.3971	36.01
19ºC	17.0622 (5.2043)**	13.6895 (5.1955)*	-0.1305 (0.0575)*	-25.8529 (10.2149)*	**3.3727**	0.5235; 9.4524	19.77
18ºC	10.7468 (3.2756)**	7.9320 (3.4450)*	-0.1199 (0.0347)***	-23.4791 (10.7603)*	**2.8148**	0.5623; 8.0286	26.19
17ºC	9.0101 (2.7922)**	6.1971 (3.0982)*	-0.1271 (0.0282)***	-22.1406 (11.3763)	**2.8130**	0.3676; 6.3115	31.22
16ºC	14.1431 (4.0529)***	11.5538 (4.0487)**	-0.1021 (0.0452)*	-25.3649 (10.1354)*	**2.5893**	0.4701; 6.8149	18.31
15ºC	21.6970 (9.6794)*	16.0500 (9.5090)	-0.2054 (0.1042)	-28.8089 (10.4058)**	**5.9170**	1.0768; 16.2034	26.94

14ºC	23.2957 (8.7497)**	23.1941 (8.2016)**	-0.0031 (0.0979)	-32.6534 (9.8079)**	0.1015	-8.4056; 8.6450	

Results shown as unstandardized coefficients (Standard Error, SE), and bias corrected (BC) 95% confidence interval (CI) of the indirect effect based on 5000 bootstraps.
BC: Bias corrected; CI: confidence interval; P_M: percentage of mediation.
Statistically significant indirect effects indicating that 0 is not in the 95% confidence interval (CI) of the indirect effect are presented in bold.
p-values indicating associations between study variables = *P<0.05, **P<0.01, ***P<0.001.

Table S2. Total, direct, and indirect effects of the simple mediation analyses investigating the mediating role of wrist temperature in the association between the number of hours exposed to a certain personal environmental temperature and standardized uptake value peak.

Independent variable	Total effect (*c*)	Direct effect (*c'*)	Path *a*	Path *b*	Indirect effect (*a*b*)	BC 95% CI Lower Upper	P_M (%)
Number of hours per day exposed to							
28ºC	-3.6344 (1.1794)**	-3.2599 (1.1593)**	0.1172 (0.0952)	-3.1947 (1.4113)*	-0.3744	-1.7238; 0.2237	
27ºC	-2.4402 (0.6799)***	-2.1041 (0.6951)**	0.1296 (0.0545)*	-2.5924 (1.4387)	**-0.3360**	-1.0857; -0.0057	13.77
26ºC	-0.8976 (0.2765)**	-0.7293 (0.2937)*	0.0702 (0.0211)**	-2.3975 (1.5152)	-0.1683	-0.4264; 0.0264	
25ºC	-1.0210 (0.4016)*	-0.7839 (0.4128)	0.0814 (0.0307)**	-2.9145 (1.5036)	**-0.2372**	-0.6959; -0.0130	23.23
24ºC	-0.4558 (0.5178)	-0.1163 (0.5213)	0.0925 (0.0384)*	-3.6683 (1.5283)*	**-0.3395**	-0.7859; -0.0673	74.49
23ºC	0.1326 (0.3487)	0.2571 (0.3384)	0.0318 (0.0265)	-3.9171 (1.4800)**	**-0.1245**	-0.3651; -0.0015	N/A
22ºC	-0.1011 (0.4022)	-0.1021 (0.3877)	-0.0003 (0.0309)	-3.7612 (1.4708)*	0.001	-0.2129; 0.2276	
21ºC	0.1960 (0.4993)	0.0180 (0.4870)	-0.0474 (0.0379)	-3.7529 (1.4872)*	0.1779	-0.0555; 0.6272	
20ºC	1.5091 (0.6993)*	1.0842 (0.7154)	-0.1373 (0.0529)*	-3.0942 (1.5139)*	**0.4249**	0.0396; 1.1213	28.16
19ºC	2.5401 (0.7171)***	2.1937 (0.7299)**	-0.1305 (0.0575)*	-2.6546 (1.4351)	**0.3463**	0.0104; 1.1236	13.63

18ºC	1.3436 (0.4626)**	1.0383 (0.4931)*	-0.1199 (0.0347)***	-2.5464 (1.5403)	**0.3053**	0.0136; 0.9144	22.72
17ºC	1.2252 (0.3901)**	0.9553 (0.4389)*	-0.1271 (0.0282)***	-2.1244 (1.6115)	0.2699	-0.0720; 0.7686	
16ºC	2.1308 (0.5563)***	1.8689 (0.5667)**	-0.1021 (0.0452)*	-2.5652 (1.4187)	**0.2619**	0.0080; 0.7930	12.29
15ºC	2.3449 (1.3674)	1.6561 (1.3661)	-0.2054 (0.1042)	-3.3535 (1.4950)*	**0.6888**	0.0485; 2.0564	29.37
14ºC	2.5672 (1.2404)*	2.5555 (1.1931)*	0.1172 (0.0979)	-3.7495 (1.4268)*	0.0117	-0.9257; 0.9111	

Results shown as unstandardized coefficients (Standard Error, SE), and bias corrected (BC) 95% confidence interval (CI) of the indirect effect based on 5000 bootstraps.
BC: Bias corrected; CI: confidence interval; P_M: percentage of mediation; N/A: non-applicable according to statistical assumptions specified previously.
Statistically significant indirect effects indicating that 0 is not in the 95% confidence interval (CI) of the indirect effect are presented in bold.
p-values indicating associations between study variables = *P<0.05, **P<0.01, ***P<0.001.

Table S3. Total, direct, and indirect effects of the simple mediation analyses investigating the mediating role of wrist temperature in the association between the number of hours exposed to a certain personal environmental temperature and metabolic activity.

Independent variable	**Total effect (*c*)**	**Direct effect (*c'*)**	**Path *a***	**Path *b***	**Indirect effect (*a*b*)**	**BC 95% CI Lower Upper**	**P_M (%)**
Number of hours per day exposed to							
28ºC	-142.2746 (49.6038)**	-123.6264 (48.0546)*	0.1172 (0.0952)	-159.1181 (58.4978)**	-18.6482	-76.7198; 13.6812	
27ºC	-92.7638 (28.7954)**	-74.7342 (29.0376)*	0.1296 (0.0545)*	-139.0875 (60.1009)*	**-18.0296**	-50.4684; -2.6810	19.44
26ºC	-34.8199 (11.6524)**	-25.4847 (12.2166)*	0.0702 (0.0211)**	-132.9461 (63.0354)*	**-9.3352**	-19.6154; -1.8476	26.81
25ºC	-41.1781 (16.8086)*	-29.0344 (17.0508)	0.0814 (0.0307)**	-149.2399 (62.1105)*	**-12.1436**	-31.4411; -2.6588	29.49
24ºC	-31.1133 (21.4178)	-15.5447 (21.3620)	0.0925 (0.0384)*	-168.2222 (62.6304)**	**-15.5686**	-32.7741; -4.8195	50.04
23ºC	-2.0202 (14.5630)	3.7930 (13.9632)	0.0318 (0.0265)	-182.8941 (61.0639)**	**-5.8132**	-15.5371; -0.0279	N/A

22ºC	-8.0621 (16.7643)	-8.1103 (15.9204)	-0.0003 (0.0309)	-180.6196 (60.3934)**	0.0482	-9.8282; 11.9435	
21ºC	6.3637 (20.8440)	-2.2449 (20.0209)	-0.0474 (0.0379)	-181.5810 (61.1410)**	8.6086	-3.0637; 29.3434	
20ºC	54.4724 (29.4166)	32.4111 (29.6347)	-0.1373 (0.0529)*	-160.658 (62.7118)*	**22.0613**	6.3974; 55.8110	40.5
19ºC	93.9165 (30.4749)**	75.3119 (30.5755)*	-0.1305 (0.0575)*	-142.6087 (60.1146)*	**18.6046**	2.8291; 52.5241	19.81
18ºC	56.6625 (19.2841)**	40.7224 (20.3314)*	-0.1199 (0.0347)***	-132.9587 (63.5043)*	**15.9400**	3.4256; 45.2335	28.13
17ºC	50.0828 (16.3261)**	34.6886 (18.1741)	-0.1271 (0.0282)***	-121.1656 (66.7343)	**15.3941**	1.9813; 38.6296	30.74
16ºC	98.7912 (22.6644)***	85.9709 (22.8526)***	-0.1021 (0.0452)*	-125.5885 (57.2095)*	**12.8203**	2.8726; 34.6266	12.98
15ºC	127.3577 (56.2649)*	95.0696 (55.6223)	-0.2054 (0.1042)	-157.2056 (60.8681)*	**32.288**	5.3280; 88.6667	25.35
14ºC	162.8793 (49.7349)**	162.3202 (46.8374)***	-0.0031 (0.0979)	-179.8669 (56.0109)**	0.5591	-40.2083; 48.2821	

Results shown as unstandardized coefficients (Standard Error, SE), and bias corrected (BC) 95% confidence interval (CI) of the indirect effect based on 5000 bootstraps.
BC: Bias corrected; CI: confidence interval; P_M: percentage of mediation; N/A: non-applicable according to statistical assumptions specified previously.
Statistically significant indirect effects indicating that 0 is not in the 95% confidence interval (CI) of the indirect effect are presented in bold.
p-values indicating associations between study variables = *P<0.05, **P<0.01, ***P<0.001.